4809 - D642
Semaphorin3A Regulates Neural Crest Migration Into the Eye During Cornea Development

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Purpose: To determine the role of lens-derived Semaphorin3A during neural crest migration into the eye.
Methods: Using in situ hybridization, we characterized the expression of *Semaphorin3A (Sema3A)* in the eye and its receptor *Neuropilin-1 (Npn-1)* by neural crest cells in the periocular region during cornea development in chick embryos. Migration of neural crest cells into the eye was tracked after lens ablation or inhibition of Sema3A in the lens using quail-chick chimera technique and immunohistochemistry.
Results: We show that the lens continuously expresses *Sema3A* during cornea development and neural crest cells express *Npn-1* in the periocular region. Interestingly, only the neural crest cells that down regulate *Npn-1* migrate into the eye to form the cornea. We also show that the lens, which immediately underlies the ectoderm, inhibits neural crest migration into the rudimentary eye since lensectomy results in premature migration and malformation of the cornea. Additionally, inhibiting of Sema3A signaling in the lens phenocopies lensectomy.
Conclusions: Our results demonstrate that lens-derived Sema3A regulates periocular neural crest migration into the eye and is necessary for the proper formation of the cornea.
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Schwann Cell Differentaition in the Embryonic Chick Cornea

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Purpose: The cornea is one of the most highly innervated tissues in the body. Neural-crest derived corneal nerve fibers encircle the cornea in the pericorneal space from Embryonic Day 6 [E6] to E9, then penetrate the anterior corneal stroma on E9, invade from the stroma into the epithelium on E12, innervate the entire corneal surface by E14, and continue to defasiculate and grow into the anterior cornea through E18. However, in adult human patients, if corneal nerves are cut for corneal transplant surgery or LASIK during adult life, regeneration of corneal nerves is very slow and incomplete. Schwann cells protect nerve fibers and can augment nerve repair following injury. In mammals, only non-myelinating Schwann cells are reported in the cornea. This study examines evidence for Schwann cell differentiation in the embryonic chick cornea.
Methods: Chick eggs were incubated from E0 at 37oC, 45% humidity. Eyes were removed on E7, E9, E12, E14, E15, E16, E19, E20; corneas plus pericorneal tissue were dissected, fixed, and stained immunohistochemically for Schwann cell proteins. In addition, corneas were dissected free of pericorneal tissue, whole-cell RNA was isolated from them with RNeasy, cDNA synthesized with iScript, and mRNA expression assessed by Real-time-PCR using iQSupermix.
Results: Antibodies for Schwann cell markers MPZ [P0], MAG [myelin-associated glycoprotein], and S100 show positive immunocytochemical staining along pericorneal nerves from E14 to E19, but no staining along nerves in the central cornea. In contrast, antibodies for Schwann cell markers Glial Fibrillary Acidic Protein [GFAP] and Schwann-cell myelin protein [SCMP] do not react with pericorneal or central corneal cells. Real-time PCR confirms increasing expressions of MPZ-like [MPZL] isoforms 1 and 3 and S100 from E9 until E20 in the cornea, but detects no expression of MAG, GFAP, or SCMP at any age. In situ hybridization experiments are in progress to determine whether cells in the central cornea are transcribing but not translating mRNAs for MPZL and S100 genes.
Conclusions: Chick central corneal Schwann cells do not synthesize Schwann-cell marker proteins, but appear to transcribe some Schwann-cell marker mRNAs.
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4811 - D644
MicroRNA Expression During Mouse Cornea Development

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Purpose: MicroRNAs (miRNA) are small, endogenously expressed non-coding RNAs that negatively regulate expression of protein-coding genes. Recent evidence has suggested a critical role for miRNAs during development and cell differentiation. To examine the function of miRNAs in mouse corneal development, we characterized the expression profiles of miRNAs in the developing mouse cornea at postnatal day (PN) 9 and in the mature cornea at 6 weeks after birth.
Methods: miRNA arrays were performed on PN9 and adult mouse (6-week-old) corneas. The validity of selected microRNA expression profiles were confirmed by qRT-PCR. A simultaneous profiling of mRNA expression was also performed by microarrays to search for candidate mRNA targets for the miRNAs.
Results: Of the 568 verified mouse miRNAs, 75 demonstrated at least 2-fold difference in expression between PN9 and adult mouse cornea. Among the top 50 abundant corneal miRNAs, 15 were differentially expressed in PN9 and adult cornea. In particular, mir-184, mir-204, mir-205, and mir-31 were up-regulated at least 5-fold in adult relative to PN9 cornea. By contrast, expression of mir-214 was down-regulated 7.5-fold in adult cornea. Many of the differentially expressed miRNAs, such as mir-31 and mir-181, have been previously shown to be involved in regulation of cell cycle progression and/or cell differentiation. Analysis of mRNA expression profile revealed that a significant number of cell cycle genes were differentially expressed (mostly down-regulated) during corneal development.
Conclusions: Our data demonstrate dynamic changes of miRNA expression in cornea after eye opening and corneal epithelial stratification. The differentially expressed miRNAs may play important roles during corneal development, particularly in regulation of cell proliferation and differentiation.
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Corneal Plasticity: Characterization of the Multipotentiality of Human Keratocytes

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Purpose: To determine the cell properties of adult human corneal keratocytes when challenged in the chick embryonic environment.
Methods: Cultured human keratocytes were injected along cranial neural crest migratory pathways in chick embryos. Human keratocytes were also cultured under various conditions and differentiated into either fibroblasts or myofibroblasts, then transplanted into the chick embryo. Migration of the injected cells was determined by immunohistochemistry using human cell-specific markers and markers of crest derivatives.
Results: Injected human keratocytes proliferated and migrated ventrally adjacent to host neural crest cells. They contributed to numerous neural crest-derived tissues including cranial blood vessels, ocular tissues, musculature of the mandibular process, and cardiac cushion tissue.
Conclusions: Adult human corneal keratocytes that have undergone terminal differentiation can be induced to form cranial neural crest derivatives when grafted into an embryonic environment.
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